Guidance for Industry

FDA recommendations for sampling and testing yellow corn and dry-milled yellow corn shipments intended for human food use for Cry9C protein residues

Final Guidance

This guidance represents FDA's current thinking on sampling and testing yellow corn and dry-milled yellow corn shipments for Cry9C protein residues. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. 21 CFR 10.115(d). The agency is now issuing final guidance for implementation in accordance with FDA's regulation on administrative practices and procedures for Good Guidance Practices, 21 CFR 10.115. (65 FR 56468; September 19, 2000).

BACKGROUND:

Recent investigations have found traces of a genetically engineered variety of corn called StarLink™ in corn-based taco shells. StarLink™ corn has been genetically modified to contain a pesticidal protein, Cry9C, which makes the corn more resistant than noncngineered varieties of corn to certain types of insects. In certain limited cases, the Cry9C protein has also been detected in corn seeds of a non-StarLink™ variety or in corn from such seeds. Unlike other varieties of genetically engineered corn, the Environmental

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Protection Agency (EPA) authorized the use of StarLink™ corn only for animal feed use, not for human food use. However, some Cry9C-containing corn was mistakenly commingled with yellow corn intended for human food use. StarLink™'s developer, Aventis S.A., in cooperation with the United States Department of Agriculture (USDA), has been buying back harvested StarLink™ corn from the year 2000 crop to prevent its introduction into the human food supply. Because some corn may have been missed in the buy-back program and because some StarLink™ corn from the 1999 crop may still be in some grain elevators, the Food and Drug Administration (FDA) is urging corn dry-milling and masa operations to screen yellow corn (and milled yellow corn in certain situations described in this document) intended for human food use to minimize the production of human food products with corn containing the Cry9C protein. Because corn containing the Cry9C pesticide is adulterated if intended for human food use, 21 U.S.C. 342(a)(2)(B), manufacturers who detect Cry9C-containing corn in any lot should divert the lot to animal feed or industrial use.

RECOMMENDED TESTING:

FDA's recommendations for testing for Cry9C-containing corn include four elements: (1) the appropriate test, (2) representative sampling, (3) appropriate analytical procedures, and (4) appropriate personnel training.

(1) Test: There are several methods currently available for detecting Cry9C-containing corn in bulk yellow corn (or milled yellow corn). FDA has been using the most sensitive

method (PCR) to test retail food products for the presence of the *cry9c* DNA, but this method is not practical for screening corn shipments in the field. For screening, FDA recommends that manufacturers use an immunoassay test that has been validated by USDA's Grain Inspection, Packers, and Stockyards Administration (GIPSA) for detecting the Cry9C protein in corn samples. The tests validated to date are the TraitCheck Bt9 Lateral Flow Test Kit (Strategic Diagnostics, Inc., Newark, DE) and the Cry9C QuickStix™ Test Kit (EnviroLogix, Inc., Portland, ME). FDA does not normally recommend tests by specific manufacturers. In this case, FDA is listing the GIPSA-validated tests that detect the Cry9C protein because the agency wants to encourage corn dry-milling and masa operations to begin testing corn as quickly as possible.

(2) Sampling: Millers and manufacturers should collect a representative sample of at least 2400 kernels from each vehicle or carrier (e.g., each rail car, barge, truck, etc.) in each incoming shipment by using the sampling protocols recommended by GIPSA. (For details, see the *Grain Inspection Handbook, Book I, Grain Sampling,* http://www.usda.gov/gipsa/reference-library/handbooks/grain-insp/grbook1/gihbk1.htm. See also GIPSA publication *Inspecting Grain: Practical Procedures for Grain Handlers* (October 1999), available at http://www.usda.gov/agency/gipsa/pubs/primer.pdf.) If the collected sample is larger than 2400 kernels, it should be mixed thoroughly and reduced to 2400 kernels for testing (see item (3) below) using an appropriate divider. (Boerner, Gamet and cargo dividers have demonstrated the ability to subdivide an original sample and have the resulting samples conform to distributions expected from a random process).

(3) Analytical procedures: A total of 2400 kernels should be tested. The 2400 kernels should be divided into subsamples for analysis, with the number of subsamples determined by the detection level for which the test has been validated (see test kit materials or contact GIPSA, 202-720-0252, www.usda.gov/gipsa). For example, for tests validated to detect StarLink™ corn when it is present at 1 kernel in 400 or 0.25% of total corn, 6 subsamples of 400 kernels each should be analyzed. For tests validated to detect StarLink™ corn when it is present at 1 kernel in 800 or 0.125% of total corn, 3 subsamples of 800 kernels each should be analyzed. Each subsample should be analyzed separately following the instructions in the test kit regarding analytical sample size and preparation procedures and adhering to detection limits validated by GIPSA.

GIPSA originally validated the TraitCheck Bt9 Lateral Flow Test Kit manufactured by Strategic Diagnostics, Inc., at a detection level of 1 in 400 kernels and the Cry9C QuickStix™ Test Kit manufactured by EnviroLogix, Inc., at a detection level of 1 in 500 kernels. GIPSA recently revalidated both test kits at a detection level of 1 in 800 kernels. Users of the Strategic Diagnostics test kit should be aware that the company has issued a new version of the test kit (part number 7000003) and that the earlier version of the kit (part number 700034) requires some protocol modifications to obtain valid results at a detection level of 1 in 800 kernels. Users of test kits with part number 700034 who wish to rely on a detection level of 1 in 800 kernels should consult with Strategic Diagnostics prior to using the kit.

As an overall example, a mill using a test validated by GIPSA to detect 1 kernel in 800 on a batch of yellow corn for which a 24-probe sample is recommended could use the following protocol: take 24 samples of corn according to GIPSA's sampling protocols, combine all 24, mix thoroughly, reduce to 2400 kernels using equipment that subdivides in a random manner, and separate the 2400 kernels into three 800-kernel subsamples. Each analytical subsample should be tested separately using a validated immunoassay kit. If any of the analytical subsamples tests positive, the entire corn lot should be diverted to animal feed use or industrial use (such as ethanol production).

(4) Personnel training: FDA also recommends that the person performing the analysis be adequately trained in the use of the test kit and be properly supervised. Employees who are performing the analysis may find the GIPSA Directive TESTING FOR STARLINK™ CORN useful.

This testing protocol is based on an analysis of the probability of rejecting corn lots containing a range of percentages of StarLink™ corn. The operating characteristic curves and tables from this analysis are provided in reference 1. The curves and tables show the likelihood that a lot of corn containing StarLink™ kernel(s) would be mistakenly accepted given different numbers of kernels tested and various levels of StarLink™ present in the lot (0% to 2.5%). For example, the table shows that a lot containing 0.19% StarLink™ corn would be rejected 99% of the time when 2400 kernels are tested according to these recommendations. A lot containing 0.29% StarLink™ corn would be rejected 99.9% of the time.

RECOMMENDATIONS FOR STORED CORN AND MEAL PRODUCTS:

During a transitional period, dry corn millers and masa producers may have inventories of stored corn that were not tested on arrival as recommended in this document. Likewise, these manufacturers/millers may have supplies of stored meal or flour products prepared from shipments of yellow corn that were not tested on arrival as recommended in this guidance. These supplies should also be tested for the Cry9C protein as recommended. For grain corn, testing (or supplemental testing if some testing has already been done) should be done on representative samples from the stored corn. For tests on milled products, FDA recommends that dry millers and masa producers take six 1-lb. subsamples from each lot of milled products and thoroughly mix each subsample individually. They should then remove an analytical sample of 120 g (equivalent to 400 kernels) from each subsample and individually extract these samples with an appropriate volume of water as specified by the test kit manufacturer. Each extracted analytical sample should then be analyzed separately following test kit instructions.

SCOPE OF THIS GUIDANCE:

These recommendations are intended to minimize the production of human food yellow corn products with corn containing the Cry9C protein, not as a guide for compliance

monitoring. As such, the recommendations may differ from the procedures outlined in FDA field assignments written for the agency's compliance monitoring activity.

In addition, the recommendations are not intended to apply to corn intended for animal feed use. Cry9C protein and the *cry9c* DNA are lawful in animal feed due to an EPA-established exemption from tolerance since the introduction of StarLink™ corn in 1998.

REFERENCE:

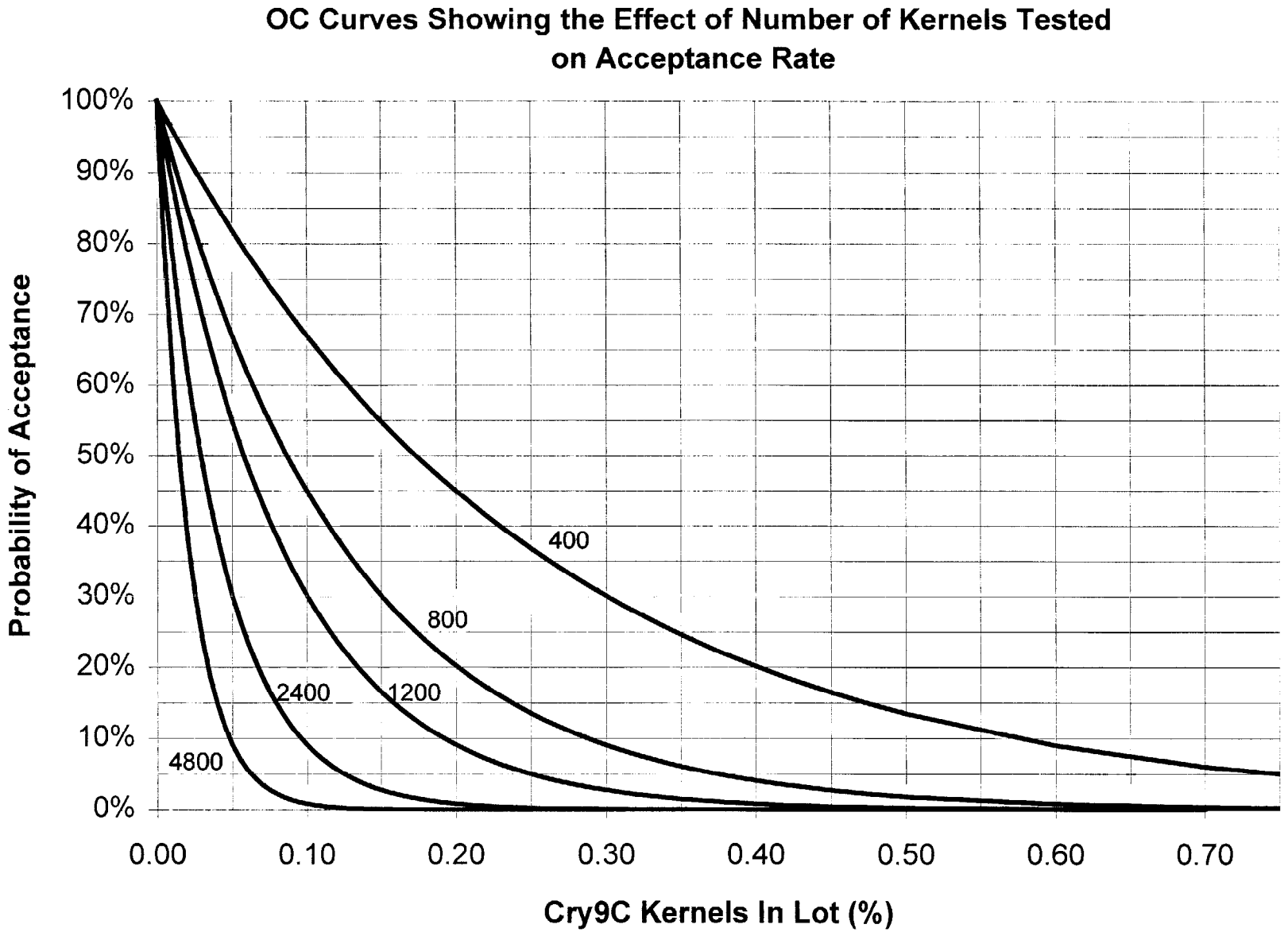
1. Sampling and testing plan scientific basis.

SAMPLING AND TESTING PLAN SCIENTIFIC BASIS

This sampling plan was developed by Dr. Thomas B. Whitaker (USDA/ARS, North Carolina State University). The operating characteristic (OC) curves, attached, were developed based on a binomial distribution that assumes a random distribution of the Cry9C-containing kernels throughout a lot of corn. This distribution is believed to reasonably reflect field conditions, provided a representative sample is obtained using the procedures in the Grain Inspection Handbook for sampling grains published by the Grain Inspection, Packers, and Stockyards Administration (GIPSA). The OC curves represent the effect on lot acceptance of testing a given number of kernels, where a lot with any positive test is rejected. The statistical treatment assumes that the variability associated with the sample preparation (i.e., grinding and homogenizing) and analytical steps are negligible or zero, and that the only variability associated with the procedure, given a specific number of tested kernels, results from the sampling step. It also assumes that no biases are associated with the sample selection process, i.e., a representative sample is obtained. The accompanying table shows the probability that a lot actually containing Cry9C kernels will test negative and be mistakenly accepted, given different numbers of tests and actual percentages of Cry9C kernels.

GIPSA has conducted studies using yellow corn to validate the test kits manufactured by Strategic Diagnostics, Inc. (Newark, DE) and EnviroLogix, Inc. (Portland, ME). GIPSA has found that the data obtained indicate that the test kits are capable of successfully detecting the presence of the Cry9C protein in corn contaminated with Cry9C-containing corn at a detection level of 1 Cry9C-containing kernel in 800, provided appropriate protocols are followed.

Manufacturers who wish to use different tests that are developed in the future should consult with GIPSA (202-720-0252, www.usda.gov/gipsa) about the validity of the tests.


OC Curves Showing the Effect of Number of Kernels Tested on Acceptance Rate
Probability of Acceptance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
400
800
1200
2400
4800
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
Cry9C Kernels In Lot (%)

Probability that a corn lot containing Cry9C kernels will test negative and be mistakenly accepted given different numbers of kernels tested and actual percentages of Cry9C kernels

	Number of kernels tested				
% StarLink	400	800	1200	2400	4800
0.005	98.020%	96.079%	94.176%	88.692%	78.662%
0.010	96.079%	92.311%	88.692%	78.662%	61.877%
0.015	94.176%	88.691%	83.526%	69.766%	48.673%
0.020	92.311%	85.213%	78.661%	61.875%	38.286%
0.025	90.483%	81.871%	74.079%	54.877%	30.115%
0.030	88.690%	78.660%	69.764%	48.670%	23.688%
0.035	86.934%	75.575%	65.700%	43.165%	18.632%
0.040	85.212%	72.610%	61.872%	38.282%	14.655%
0.045	83.524%	69.762%	58.268%	33.951%	11.527%
0.050	81.869%	67.025%	54.873%	30.110%	9.066%
0.055	80.247%	64.396%	51.676%	26.704%	7.131%
0.060	78.657%	61.869%	48.665%	23.683%	5.609%
0.065	77.099%	59.442%	45.829%	21.003%	4.411%
0.070	75.571%	57.110%	43.158%	18.626%	3.469%
0.075	74.073%	54.869%	40.643%	16.519%	2.729%
0.080	72.606%	52.716%	38.275%	14.649%	2.146%
0.085	71.167%	50.647%	36.044%	12.992%	1.688%
0.090	69.756%	48.659%	33.943%	11.521%	1.327%
0.095	68.374%	46.750%	31.965%	10.217%	1.044%
0.100	67.019%	44.915%	30.101%	9.061%	0.821%
0.105	65.690%	43.152%	28.347%	8.035%	0.646%
0.110	64.388%	41.458%	26.694%	7.126%	0.508%
0.115	63.112%	39.831%	25.138%	6.319%	0.399%
0.120	61.861%	38.267%	23.672%	5.604%	0.314%
0.125	60.634%	36.765%	22.292%	4.969%	0.247%
0.130	59.432%	35.322%	20.992%	4.407%	0.194%
0.135	58.254%	33.935%	19.768%	3.908%	0.153%
0.140	57.098%	32.602%	18.615%	3.465%	0.120%
0.145	55.966%	31.322%	17.530%	3.073%	0.094%
0.150	54.856%	30.092%	16.508%	2.725%	0.074%
0.155	53.769%	28.911%	15.545%	2.416%	0.058%
0.160	52.702%	27.775%	14.638%	2.143%	0.046%
0.165	51.657%	26.684%	13.784%	1.900%	0.036%
0.170	50.632%	25.636%	12.980%	1.685%	0.028%
0.175	49.628%	24.629%	12.223%	1.494%	0.022%
0.180	48.644%	23.662%	11.510%	1.325%	0.018%
0.185	47.679%	22.733%	10.839%	1.175%	0.014%
0.190	46.733%	21.840%	10.206%	1.042%	0.011%
0.195	45.806%	20.982%	9.611%	0.924%	0.009%
0.200	44.897%	20.157%	9.050%	0.819%	0.007%
0.210	43.133%	18.605%	8.025%	0.644%	0.004%
0.220	41.438%	17.171%	7.115%	0.506%	0.003%

	Number of kernels tested				
% StarLink	400	800	1200	2400	4800
0.230	39.810%	15.848%	6.309%	0.398%	0.002%
0.240	38.245%	14.627%	5.594%	0.313%	0.001%
0.250	36.742%	13.500%	4.960%	0.246%	0.001%
0.260	35.298%	12.459%	4.398%	0.193%	0.000%
0.270	33.910%	11.499%	3.899%	0.152%	0.000%
0.280	32.577%	10.612%	3.457%	0.120%	0.000%
0.290	31.296%	9.794%	3.065%	0.094%	0.000%
0.300	30.065%	9.039%	2.718%	0.074%	0.000%
0.310	28.883%	8.342%	2.409%	0.058%	0.000%
0.320	27.747%	7.699%	2.136%	0.046%	0.000%
0.330	26.655%	7.105%	1.894%	0.036%	0.000%
0.340	25.607%	6.557%	1.679%	0.028%	0.000%
0.350	24.599%	6.051%	1.489%	0.022%	0.000%
0.360	23.631%	5.584%	1.320%	0.017%	0.000%
0.370	22.701%	5.154%	1.170%	0.014%	0.000%
0.380	21.808%	4.756%	1.037%	0.011%	0.000%
0.390	20.950%	4.389%	0.919%	0.008%	0.000%
0.400	20.125%	4.050%	0.815%	0.007%	0.000%
0.410	19.333%	3.738%	0.723%	0.005%	0.000%
0.420	18.572%	3.449%	0.641%	0.004%	0.000%
0.430	17.840%	3.183%	0.568%	0.003%	0.000%
0.440	17.138%	2.937%	0.503%	0.003%	0.000%
0.450	16.463%	2.710%	0.446%	0.002%	0.000%
0.460	15.814%	2.501%	0.396%	0.002%	0.000%
0.470	15.192%	2.308%	0.351%	0.001%	0.000%
0.480	14.593%	2.130%	0.311%	0.001%	0.000%
0.490	14.018%	1.965%	0.275%	0.001%	0.000%
0.500	13.466%	1.813%	0.244%	0.001%	0.000%
0.600	9.006%	0.811%	0.073%	0.000%	0.000%
0.700	6.021%	0.363%	0.022%	0.000%	0.000%
0.800	4.024%	0.162%	0.007%	0.000%	0.000%
0.900	2.688%	0.072%	0.002%	0.000%	0.000%
1.000	1.795%	0.032%	0.001%	0.000%	0.000%
1.100	1.198%	0.014%	0.000%	0.000%	0.000%
1.200	0.799%	0.006%	0.000%	0.000%	0.000%
1.300	0.533%	0.003%	0.000%	0.000%	0.000%
1.400	0.355%	0.001%	0.000%	0.000%	0.000%
1.500	0.237%	0.001%	0.000%	0.000%	0.000%
1.600	0.158%	0.000%	0.000%	0.000%	0.000%
1.700	0.105%	0.000%	0.000%	0.000%	0.000%
1.800	0.070%	0.000%	0.000%	0.000%	0.000%
1.900	0.047%	0.000%	0.000%	0.000%	0.000%
2.000	0.031%	0.000%	0.000%	0.000%	0.000%
2.100	0.021%	0.000%	0.000%	0.000%	0.000%
2.200	0.014%	0.000%	0.000%	0.000%	0.000%
2.300	0.009%	0.000%	0.000%	0.000%	0.000%
2.400	0.006%	0.000%	0.000%	0.000%	0.000%
2.500	0.004%	0.000%	0.000%	0.000%	0.000%